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VITAMIN A POTENCY OF IRRADIATED MILK

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(FROM THE RESEARCH LABORATORIES OF THE DRY MILK COMPANY, NEW YORK)

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Numerous investigators have shown that the antirachitic potency of milk can be increased by exposure to the rays of the quartz mercury vapor lamp. However, the desirability of this procedure for enhancing the nutritive properties of milk serving as the sole dietary of the infant, has been questioned, because certain experimental evidence has indicated that the vitamin A of milk is destroyed by irradiation. Titus, Hughes, *et al.* (1) claim that this vitamin is materially decreased in milk irradiated for 20 minutes[1] at a distance of 18 to 20 inches from the lamp. Drummond (2) has likewise pointed out the possibilities of vitamin A destruction in irradiated milk, and he also calls attention to the fact that important, and presumably unfavorable changes are brought about by irradiation as manifested by the fact that milk exposed for as short a time as 5 minutes is highly unpalatable. Spinka (3) states that vitamin A is not destroyed by an exposure of 20 minutes to ultra-violet light, but that the ozone generated by the lamp brings about detrimental results. He states that these results are not evident when air is excluded from the product during irradiation. Steenbock and Coward (4) state that according to their observations, both vitamins A and B have shown marked stability to ultra-violet light. During investigations conducted at this laboratory on milk activated antirachitically with the ultra-violet light, which have extended over a period of 2 to 3 years, observations have not indicated a perceptible destruction of vitamin A in milk irradiated to a degree which imparts marked prophylactic properties for rickets to the product. From these observations as well as those to which reference has been

[1] Personal communication supplementing the published data.

made, it appears that there is no conclusive evidence indicating that irradiation *per se* destroys vitamin A. It is conceivable that the destructive effect reported by certain investigators has been due to prolonged exposure whereby reactions of a detrimental character have been brought about, such reactions being of a secondary and irrelevant nature in so far as antirachitic activation of the product is concerned.

Data as yet unpublished but now in press, have shown that under suitable conditions, milk, either in liquid or dry form, may be activated to a marked degree by a brief period of exposure. The bone-forming properties of the milk irradiated in this manner are best shown in Table I[2] which summarizes the averages obtained from 217 parallel determinations made with the irradiated and non-irradiated milk.[3]

Since the dry milk in question, irradiated either before or after

TABLE I.

Percentage Ash in Long Bones of White Rats Fed Variable Quantities of Irradiated and Non-Irradiated Reconstituted Dry Milk.

Rachitic ration with:	2 cc. daily.	4 cc. daily.	7 cc. daily.	9 cc. daily.	15 cc. daily.
Non-irradiated dry milk.....	43.5	45.5	47.2	52.8	55.6
Irradiated dry milk.....	49.0	53.0	55.1	58.2	60.0

desiccation, was found to possess increased calcifying properties as shown in Table I, the irradiated product has been subjected to further critical studies for the purpose of determining whether or not measurable vitamin A destruction could be detected. The irradiation technique as applied to the product was as follows: For treatment before desiccation the milk was successively exposed in thin films at variable distances from a quartz mercury vapor lamp of the Hanovia type. The minimum exposure distance from the source of light was not less than 12 inches and the maximum distance did not exceed 4 feet. Although the data showing the exact period of exposure at the different distances

[2] These data are included in an article which will appear in a forthcoming issue of the *Am. J. Dis. Child*, in press.

[3] The product under consideration was a milk containing 12 per cent fat on the dry basis and is known commercially as Dryco brand dry milk.

from the source of light are not available, it is estimated that the total exposure period was for only a period of seconds, and at any rate not over 1 minute. For treatment after desiccation, the finely pulverized dry milk was likewise exposed successively at variable distances from a quartz mercury vapor lamp of the Cooper Hewitt type. During the exposure period the powder was subjected to the action of an especially devised agitating apparatus in order to disperse the powder particles thoroughly, thereby permitting an exposure of a very large fraction of the surface area of each particle to the rays of the lamp. The exposure distances were substantially the same as for liquid milk irradiated, but the average exposure period is estimated to vary from 30 seconds to 3½ minutes.

EXPERIMENTAL.

For the experimental work reported herein, the starvation or vitamin A depletion method was used. The test animals were vigorous white rats selected from our stock colony, 25 to 28 days old at the time they were used for experimentation. During the preexperimental period they were allowed to consume the stock colony ration. While on the test rations they were kept in individual cages with screened bottoms. The basal ration consisted of casein, 18 parts; Salt Mixture 40 (5), 4 parts; powdered agar-agar, 2 parts; dry yeast, 6 parts; and dextrin, 70 parts. The casein was freed from vitamin A by extraction 10 days with alcohol and subsequent heating for 1 week at 96°C.

In order to eliminate difficulties in interpreting the results, in so far as the status of vitamin A and the antirachitic factor were concerned, recourse was made to three different procedures. For one group of test animals no supplementary source of the antirachitic factor was furnished during the vitamin A depletion period; the only antirachitic factor furnished throughout the entire course of the experimental feeding period was that contained in the irradiated or non-irradiated test milks. In the various accompanying charts this group is always designated by a lot number which begins with the figure 1. For the second group the antirachitic factor was supplied throughout the test period by irradiation of the basal ration for 20 minutes at 26 inches from the lamp. The antirachitic factor supplied in this way was therefore

available during the starvation period and supplemented that contained in the experimental milks fed after the vitamin A of the body had been exhausted. In the accompanying charts this group is always designated by a lot number which begins with the figure 2. For the third group the antirachitic factor was supplied throughout the experimental period by direct irradiation of the animals for 3 minutes 6 days a week. In our experience this period of exposure has been found to be just as satisfactory as a longer period. In the accompanying charts this group is always designated by lot numbers which begin with the figure 3.

Vitamin A depletion of the body store was determined by cessation of growth or an occurrence of ophthalmia; both usually occurred simultaneously. The manifestation of ophthalmia can be considered as positive indication of vitamin A depletion, whereas cessation of growth only, may possibly be due to a deficiency of the antirachitic factor, unless this factor is supplied by direct body irradiation or by irradiation of the basal ration. By providing in the experimental plan for different series in which there was an absence of the antirachitic factor in one instance (Series 1), and an adequacy of this factor in others (Series 2 and 3), conclusions regarding the end-point of the vitamin A starvation period are quite definite. For illustration, in those instances where the antirachitic factor supplemented the basal ration and cessation of growth resulted without concurrent ophthalmia, the cessation of growth must have been due to depletion of the body store of vitamin A, whereas, if cessation of growth resulted without the manifestation of ophthalmia in those instances where no supplementary antirachitic factor was furnished in addition to the basal ration it is not definitely known whether the cessation of growth was due to the absence of vitamin A or the absence of the antirachitic factor. With the three series as provided for in the present experimental plan definite assurance regarding the time of vitamin A depletion is possible.

When the growth rate of the individual rats declined, or when ophthalmia occurred, the milk feeding of the test animals was started. The milk as obtained at the laboratory was in dry form, part of which had been irradiated before desiccation and part after desiccation, as described. At the same time the irradiated samples were obtained some of the same milk non-irradiated was

also obtained. All these milks were obtained during the winter season. The samples irradiated before desiccation and the non-irradiated check were obtained during the month of January, 1926; the samples irradiated after desiccation and the non-irradiated check samples were obtained early in April of the same year. The milks were reconstituted to the original fluid milk basis and 5 and 10 cc. quantities of each were fed daily. Since the milk, when reconstituted to the liquid basis, contained about 1.2 per cent of butter fat, these quantities represented approximately 1.65 cc. and 3.3 cc., respectively, of fluid milk of average fat content.

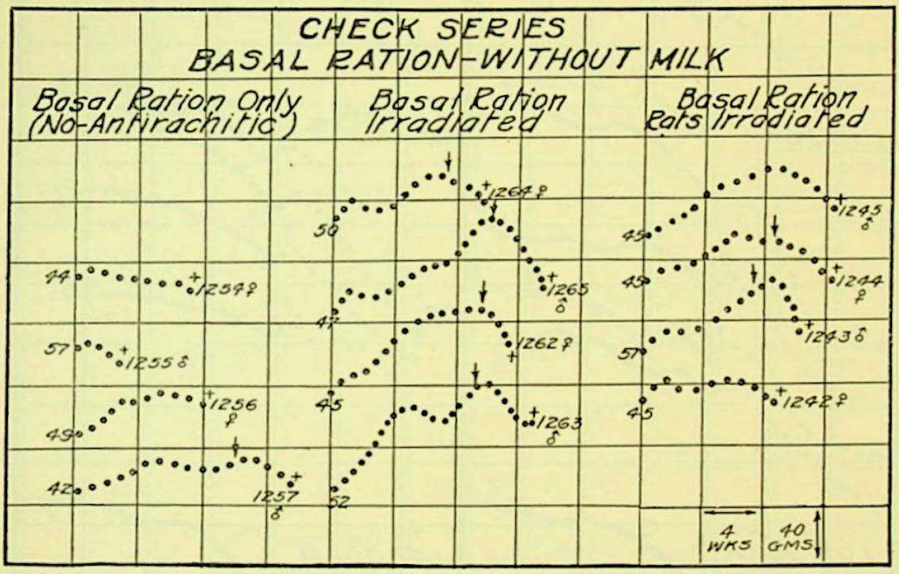

Chart 1.

Computed on the butter fat basis, therefore, the quantities fed represented marginal amounts which might be expected to furnish an adequacy of vitamin A, providing there had been no destruction of this factor during desiccation or irradiation. It has already been shown that the desiccation of milk by the Just roller process (the method used for drying the milk under consideration) does not cause a measurable destruction of vitamin A (6).

The growth curves recorded in the accompanying charts are presented without selection in order to allow free interpretation of the results. Chart 1 shows the growth curves of the control

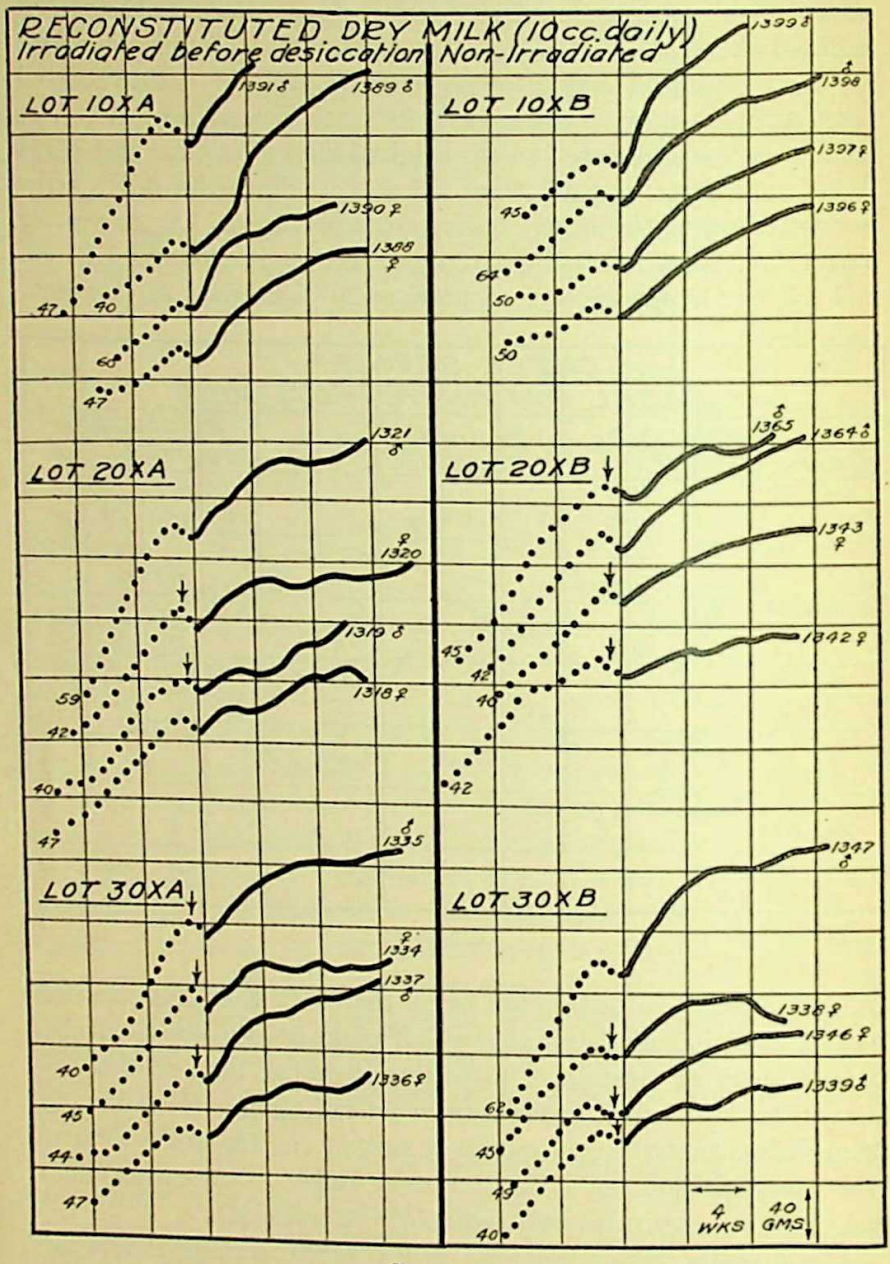
RECONSTITUTED DRY MILK (10cc. daily)
Irradiated before desiccation
Non-Irradiated
LOT 10XA
LOT 10XB
LOT 20XA
LOT 20XB
LOT 30XA
LOT 30XB
4 WKS
40 GMS

CHART 2.

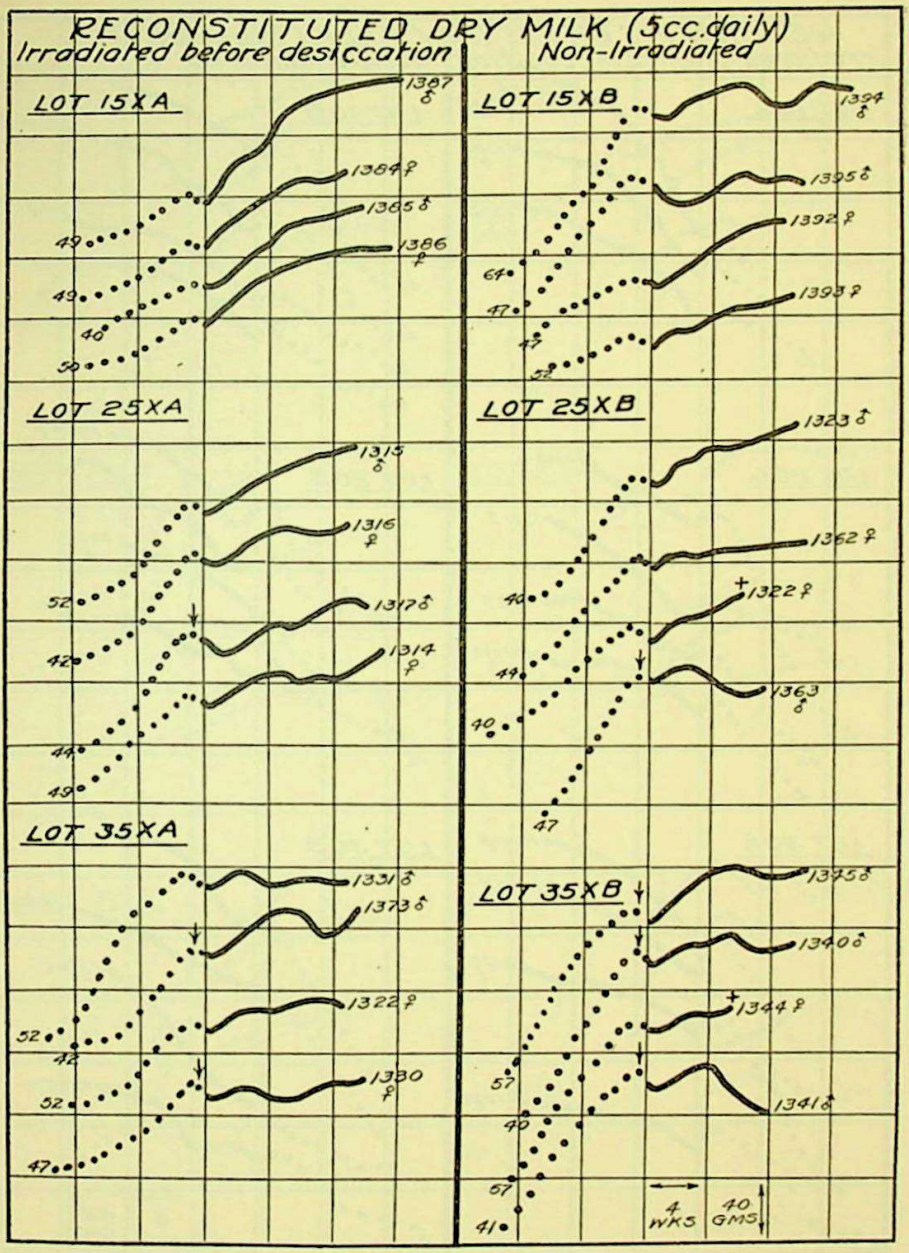
RECONSTITUTED DRY MILK (5cc daily)
Irradiated before desiccation
Non-Irradiated
LOT 15XA
LOT 15XB
LOT 25XA
LOT 25XB
LOT 35XA
LOT 35XB
4 WKS
40 GMS

Chart 3.

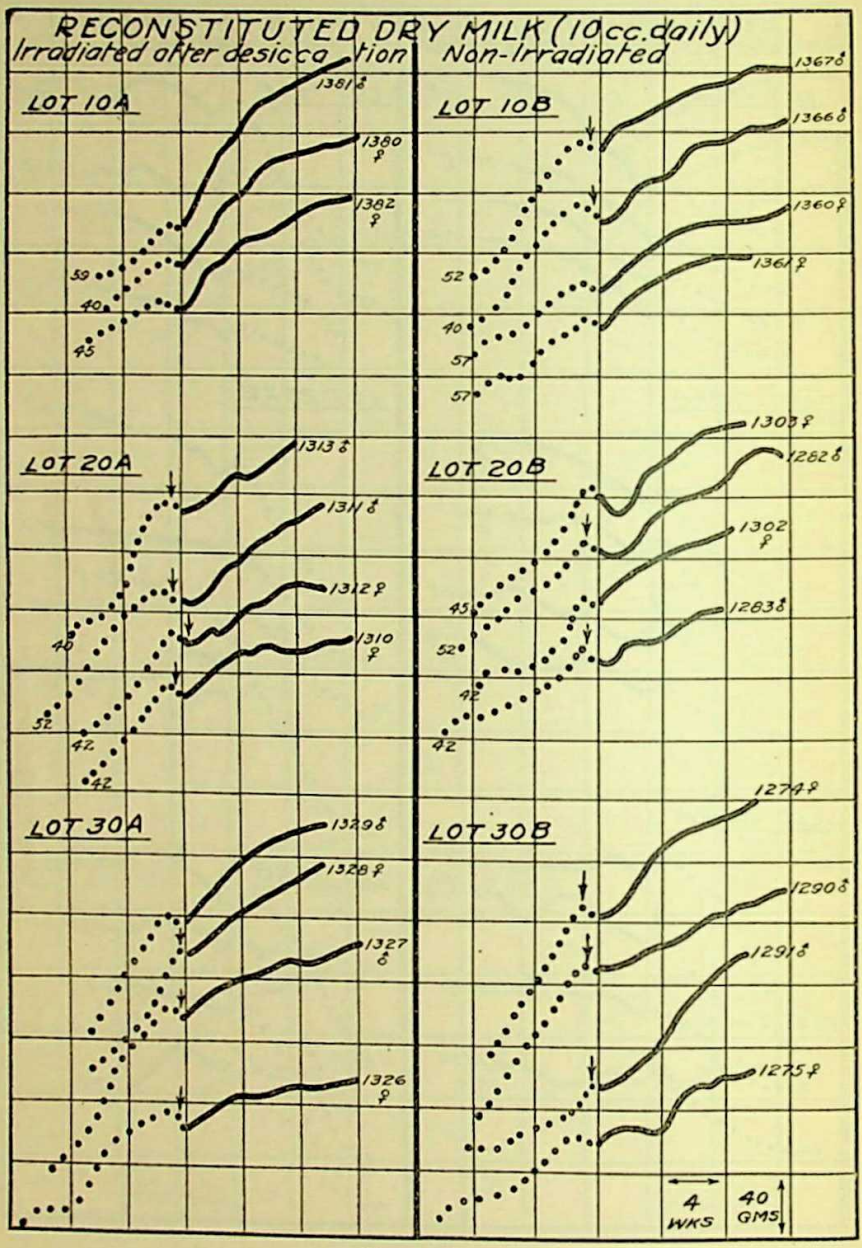
RECONSTITUTED DRY MILK (10cc. daily)
Irradiated after desiccation
Non-Irradiated
LOT 10A
LOT 10B
LOT 20A
LOT 20B
LOT 30A
LOT 30B
4 WKS
40 GMS

Chart 4.

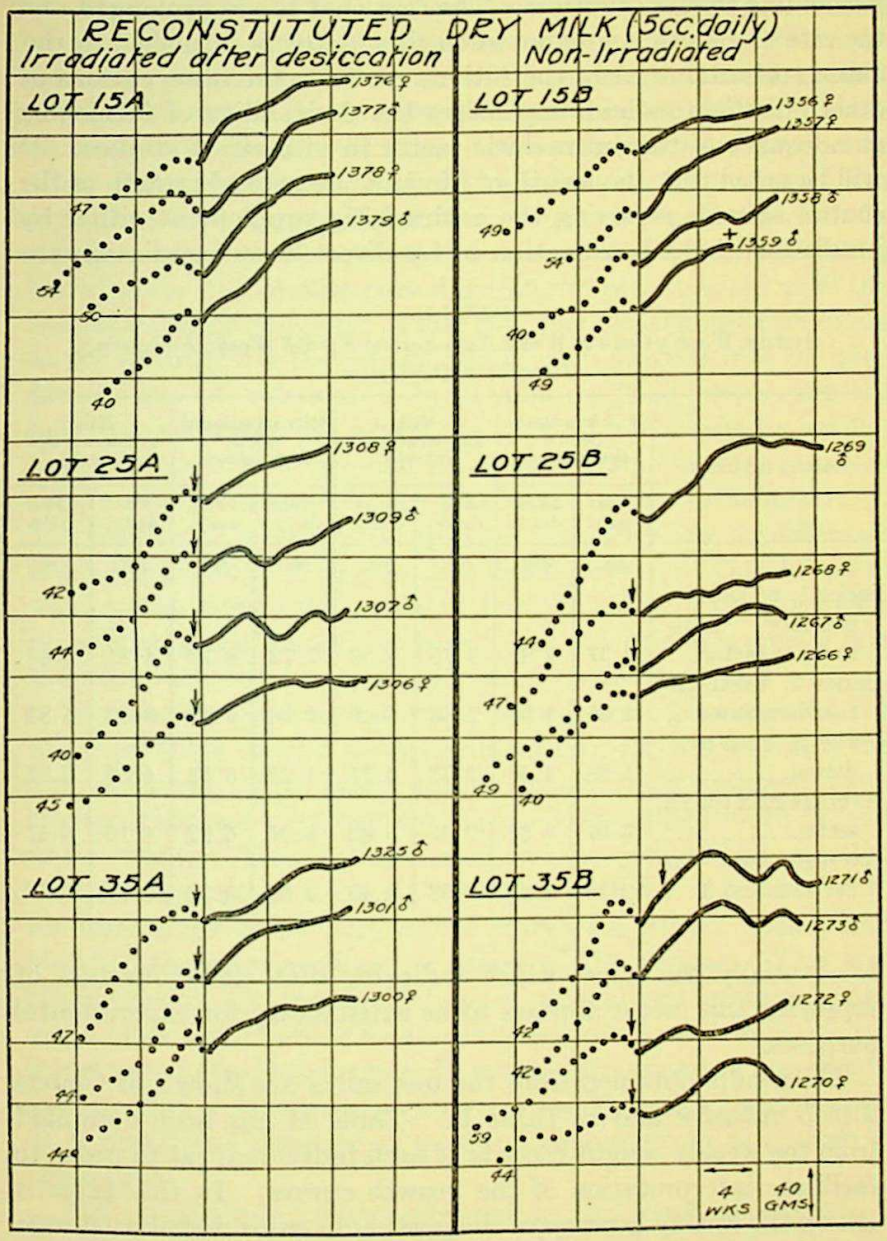
RECONSTITUTED DRY MILK (5cc daily)
Irradiated after desiccation
Non-Irradiated
LOT 15A
1376♀
1377♂
1378♀
1379♂
47
54
50
40
LOT 15B
1356♀
1357♀
1358♂
1359♂
49
54
40
49
LOT 25A
1308♀
1309♂
1307♂
1306♀
42
44
40
43
LOT 25B
1269♂
1268♀
1267♂
1266♀
44
47
49
40
LOT 35A
1325♂
1301♂
1300♀
42
44
44
LOT 35B
1271♂
1273♂
1272♀
1270♀
42
42
39
44
4 WKS
40 GMS

Chart 5.

animals and reveals the influence of the antirachitic factor supplementing the basal ration. The fact that life is prolonged and the rate of growth is greater when this factor is furnished in the absence of vitamin A in the ration, confirms the observations of other investigators and emphasizes the desirability of furnishing an adequacy of the antirachitic factor in vitamin A studies. It will be noted that the length of life and the rate of growth of the control animals receiving the antirachitic supplement either by irradiation of the basal ration or by direct body irradiation are

TABLE II.

Average Weekly Gain of White Rats during First 8 Weeks Following Vitamin A Depletion.

Amount fed daily.	Milk irradiated before desiccation.		Milk non-irradiated (check).		Milk irradiated after desiccation.		Milk non-irradiated (check).	
	5 cc. daily.	10 cc. daily.	5 cc. daily.	10 cc. daily.	5 cc. daily.	10 cc. daily.	5 cc. daily.	10 cc. daily.
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Series 1, no supplementary antirachitic factor....	6.37	8.87	2.75	8.59	7.72	9.70	4.81	5.81
Series 2, basal ration irradiated....	2.65	3.94	2.33	4.09	2.90	5.25	3.50	5.83
Series 3, rats irradiated.....	1.68	4.62	2.33	4.77	4.25	5.52	4.00	6.87
Average gain for all series.....	3.57	5.81	2.47	5.82	4.96	6.82	4.10	6.17
Average gain for Series 2 and 3.....	2.16	4.28	2.33	4.43	3.57	5.38	3.75	6.35

substantially the same, which indicates that either method of supplying this factor appears to be satisfactory for experimental purposes.

The results obtained from the test milks are shown in Charts 2 to 5 inclusive and in Table II. Table II has been compiled from the weekly weight records of each individual rat in order to facilitate interpretation of the growth curves. In this table is shown the weekly average of the maximum gains distributed over the first 8 weeks of the milk feeding period.

An inspection of the data shows that the gain resulting from the

feeding of 10 cc. quantities is invariably greater than when 5 cc. were fed. This is to be expected in view of the marginal quantities of vitamin A carried by these amounts, particularly the 5 cc. quantity. The significant item for comparison, however, is that there is substantially no difference in the rate of growth of those animals receiving the irradiated and non-irradiated product in those series where an adequacy of the antirachitic factor was furnished from the very beginning of the experimental period. In the first series where no antirachitic factor was furnished during the vitamin A depletion period, greater growth resulted when the irradiated milk was fed. While this may be attributed primarily to the antirachitic factor, the relationship also confirms the non-destruction of vitamin A by irradiation, and emphasizes the nutritive enhancement which has accrued to the milk by irradiation. In the other two series the comparable average gains are substantially the same for the irradiated and non-irradiated milk. The particular exception being in one instance where the difference is 1.3 gm. in favor of the non-irradiated product. This difference is accounted for by the record of Rat 1291 which gained but very little during the starvation period but did gain at a greater rate than the others of the same group when the milk was fed.

The weekly food consumption records were kept for each rat but since these records show no unexpected relationships they are not included. The basal ration consumption followed quite closely the trend of the growth curves, decreasing toward the end of the vitamin A starvation period and increasing again when the milk feeding was started. Coincident with the disinclination to eat during the latter part of the starvation period, kidney and bladder disorders occurred in many of the animals. This was characterized by the formation of crystallized masses in the urine after excretion; it was usually remedied when milk was fed irrespective of whether the milk was the irradiated or non-irradiated product. It is possible that this condition is a manifestation of a functional disorder more or less characteristic of vitamin A deficiency.

The ophthalmia which appeared as the result of vitamin A depletion was cured in all instances when milk was fed. Usually this condition was completely rectified within 10 days to 2 weeks (see Figs. 1 and 2). Some of the animals receiving the 5 cc.

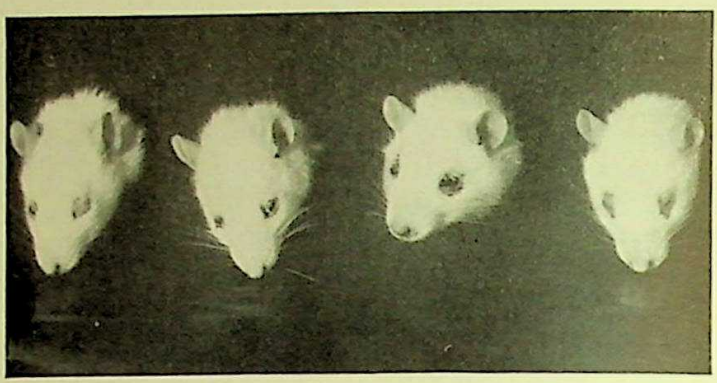

FIG. 1.

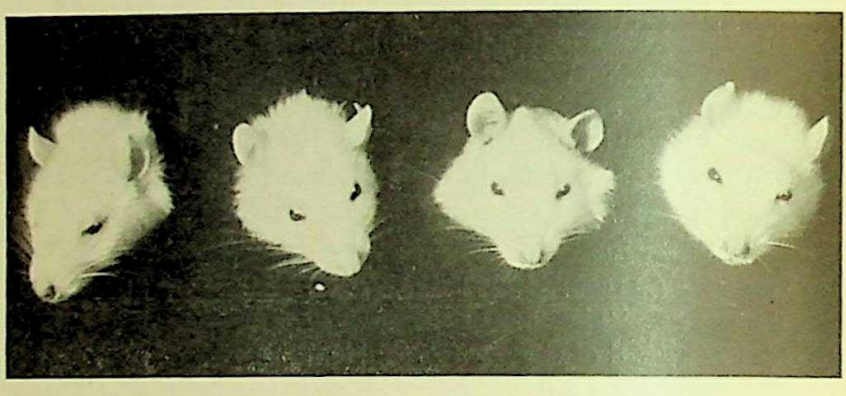

FIG. 2.

FIGS. 1 AND 2. In Fig. 1 are shown typical rats at the time of vitamin A depletion, all with severe ophthalmia; in Fig. 2 are shown the same rats 10 days after receiving 10 cc. daily quantities of reconstituted irradiated dry milk. Complete cure has been brought about in each case.

quantities showed a subnormal condition of the eyes after a milk feeding period of 10 to 12 weeks. This condition was noted with equal frequency among animals receiving the irradiated and non-irradiated product. It presumably indicates the marginal protective quantities of vitamin A in the 5 cc. of the low fat milk.

SUMMARY.

Milk irradiated either in dry or liquid form, for short periods under suitable conditions, which had been previously shown to have had imparted to it marked bone-forming properties and prophylactic qualities for rickets, shows, according to the data presented, no evidence of vitamin A destruction, or other toxic effects.

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